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(57) Abstract

Delivery of fuel and cofactors augments ATP production in cells, and mitigates damages in ischemic or metabolically impaired tissues. The processes may be particularly effective in acute or chronic ischemic conditions, for reversing anesthesia, for treating diabetes, for producing or preventing coma due to lack of fuel of ATP, for reversing processes of aging, as dietary supplements, as performance enhancers, for example, in sports, for tissue transplantation and other surgery, and for cold storage or cryopreservation of tissues such as organs.

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AUGMENTED ATP PRODUCTION

BACKGROUND OF THE INVENTION

The production of ATP (adenosine triphosphate) is essential for cellular energy metabolism. Cellular ATP production can take place either during glycolysis or during mitochondrial processing of the pyruvate which results from glycolysis. Glycolysis is therefore necessary for the production of ATP from sugars under both aerobic and anaerobic conditions. Since ATP is essential to continued cell function, when aerobic metabolism is slowed or prevented by lack of oxygen, for example, by hypoxia or anoxia due to anemia, deficient oxygen supply or ischemia, anaerobic pathways for producing ATP are stimulated and become critical for maintaining viability.

The first stage of glycolysis involves the transformation of glucose into fructose 1,6-bisphosphate (FBP). This transformation requires using two molecules of ATP per molecule of glucose converted to FBP. Exogenously supplied FBP is thus a more energetically advantageous substrate than glucose.

Production of pyruvate from glucose or FBP yields four molecules of ATP per molecule of glucose. Thus, in anaerobic metabolism, a molecule of FBP has a net yield of twice the number of ATP molecules as does a molecule of glucose.

Another advantage of using FBP over using glucose in metabolism results from decreased pH caused by ischemia or hypoxia. When aerobic pathways are not available to metabolize pyruvate, pyruvate and lactate (and their acids) accumulate in cells, causing an increased concentration of hydrogen ions (a decreased pH). Conversion of glucose to FBP is inhibited by a low pH, but the conversion of FBP to pyruvate is not strongly pH dependent. Glucose transport into cells is also inhibited by a low pH.

U.S. Patents Nos. 4,596,095, 4,703,040, 4,757,052 and 5,039,665 to Markov recognize advantages of FBP as an alternate energy source to glucose for conditions in which

ischemic or hypoxic conditions have compromised ATP production.

In order to complete the conversion of pyruvate to CO_2 and water, thereby producing large amounts of ATP, oxygen is required. Pyruvate therefore does not act as an energy source during anoxia, for example, anoxia resulting from ischemia.

U.S. Patent No. 5,395,822 to Izumi has recently described that the presence of pyruvate during ischemia allows the hippocampus to retain better integrity after reoxygenation than does the presence of glucose. One explanation of this phenomenon is that the decreased pH resulting from the ischemia inhibits metabolism of glucose to pyruvate. However, when oxygen is re-introduced, the presence of exogenous pyruvate provides a pool of pyruvate for use by the mitochondria independent of the acid inhibition of glycolysis.

Both FBP and pyruvate have been found beneficial in restoring ATP generation capabilities following periods of ischemia. However, alternate therapies for regenerating ATP production capability could provide critically needed flexibility for a clinician treating idiosyncratic patients, and alternate therapies that work better than FBP or pyruvate could save and improve lives. Also because various cell types and the blood brain barrier will vary in permeabilities to different molecules, substrates other than FBP and pyruvate are desirable. For example, FBP has been shown to be ineffective for cerebral resuscitation after severe cerebral oxygen deprivation despite salutary effects on the heart in a hemorrhagic shock model. Agents better able to penetrate the brain may overcome this deficiency.

All references cited herein are hereby incorporated herein in their entirety.

SUMMARY OF THE INVENTION

This invention relates to the augmentation or maintenance of cellular ATP levels. The ATP augmenting chemicals and methods of the present invention stimulate

glycolysis and mitochondrial metabolism by providing fuel and/or cofactors necessary for the production of ATP either anaerobically or aerobically. These chemicals can be delivered to a tissue, for example, an organ or an intact organism such as a person, e.g., in vitro or in vivo, to maintain or provide for ATP synthesis when aerobic ATP synthesis is or has been compromised. The compounds and uses of the invention may be useful for reducing or preventing tissue damage in acute or chronic ischemia, for reversing anesthesia, for treating diabetes, for reversing or preventing coma due to lack of fuel or ATP, for reversing processes of aging, for use as dietary supplements, or as performance enhancers, for example, in sports, for tissue transplantation and other surgery, involving ischemic and/or hypoxic or anoxic conditions and for cold storage or cryopreservation of organs.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the relationship between ATP content and a functional measure of cell viability, the K/Na ratio, after exposure to cryoprotectants.

Figure 2 shows that the presence of high extracellular NAD^+ concentrations preclude accurate measurement of intracellular ATP.

Figure 3 is a summary of selected results of two experiments in which kidney slices were incubated in the presence of glycolytic intermediates during anoxia.

Figure 4 is a summary of the results of incubating rabbit renal cortical slices in the presence of glycolytic intermediates and anoxia and then restoring oxygen and glucose as the only substrate.

Figure 5 presents the effects of CoA and NAD^+ on the viability of kidney slices stored for several days near 0°C.

Figure 6 shows the ineffectiveness of G3P and PEP in reversing the effects of cryoprotective agents.

Figure 7 shows the ineffectiveness of 1 mM dichloroacetate in reversing the effects of cryoprotective agents.

Figure 8 shows the moderate effectiveness of G3P plus NAD+ plus 4 mM dichloroacetate in reversing the effects of cryoprotective agents.

Figure 9 shows the moderate effectiveness of NAD+ and the strong effectiveness of Coenzyme A in reversing the effects of cryoprotective agents.

Figure 10 shows the effectiveness of Coenzyme A in reducing damage in kidney slices cooled without freezing to -30°C.

Figure 11 shows the ineffectiveness of Coenzyme A in reversing cryoprotectant effects when Coenzyme A is introduced simultaneously with the cryoprotectant.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Inter alia, the instant invention solves problems associated with restoring normal metabolism after metabolism has been slowed or stopped. Metabolism can be slowed or stopped in several ways including chemical, for example, decreased pH, or more specific inhibitors such as anesthetics, deprivation of oxygen, for example, by anemia, carbon monoxide poisoning, slowing or stopping circulation, and decreased temperature. The invention provides methods and compositions for maintaining or restoring metabolism during or following such a metabolic crisis.

The metabolism of glucose to carbon dioxide and water has been studied extensively and chemical compounds in the pathway are well known. To date, however, the chemical compounds of the present invention have not been contemplated for the purposes of the present invention.

G3P

The invention embodies administration of glyceraldehyde 3-phosphate (G3P) to a tissue as a glycolytic substrate. G3P has a lower molecular mass and less charge than FBP. G3P therefore can penetrate cells more rapidly than FBP. Another advantage of G3P over FBP is that G3P is farther along in glycolysis than FBP, and therefore may be effective when FBP would be ineffective due to inhibition of any of the bypassed enzymes necessary

for conversion of FBP to G3P, yet the G3P made from FBP retains all the ATP-generating potential of the parent molecule.

Dihydroxyacetone phosphate, the metabolic intermediate immediately upstream of G3P, and 1,3-diphosphoglycerate, the metabolic intermediate immediately downstream of G3P, are too unstable to be of practical use. Since each FBP molecule is split to produce two G3P molecules, twice as many molecules (but roughly the same number of grams) of G3P as FBP should nominally be present when using G3P to replace FBP in restoring metabolism. As described below, however, G3P is often comparable to FBP when given in doses no greater than or even less than doses of FBP.

NAD$^+$

Another chemical required for the metabolism of FBP or G3P to ATP is the cofactor, NAD$^+$. NAD$^+$ is usually present at low concentrations within the cell. During metabolism G3P is oxidized to 3-phosphoglycerate (3-PG) as NAD$^+$ is reduced to NADH. In order to regenerate NAD$^+$, NADH reduces pyruvate to lactate. The regenerated NAD$^+$ is then available to oxidize G3P to keep the ATP generation process functioning.

This NADH/NAD$^+$ cycle has three deleterious results. First, lactic acid, a metabolic inhibitor, accumulates within the cell as a result of reduction of pyruvate by NADH. Second, pyruvate, which could otherwise be used to provide ATP, is made temporarily unavailable due to conversion to lactate. Third, when all of the accumulated lactate is suddenly converted into pyruvate upon reoxygenation, the NAD$^+$ consumed in the process could temporarily limit glycolysis.

The invention provides compositions and methods for delivering NAD$^+$ to tissues, for example in an organ or person. Delivery can be accomplished through, for example, a perfusing or bathing solution, an IV solution, an injectable supplement or a food supplement containing

the composition being provided to the afflicted area or person in liquid or solid forms.

Supplementing cells with NAD^+ has at least the following advantages: first, since NAD^+ is an end product of lactate formation, supplementation with NAD^+ will reduce lactate production during hypoxia and therefore make more pyruvate available to the mitochondria immediately upon reoxygenation; second, by minimizing the lactate pool, the depletion of cytoplasmic NAD^+ upon reoxygenation due to the oxidation of lactate back to pyruvate will be minimized, thereby conserving NAD^+ for the promotion of glycolysis; third, the NADH accumulated because of reduced conversion to NAD^+ by lactate production is immediately available for oxidation by the electron transport chain when oxygen is reintroduced; fourth, the conserved NADH may also participate in anabolic reactions; fifth, since NAD^+ is used in multiple cell pathways and may be depleted under ischemic and other conditions, supplementing with NAD^+ will correct any such deficiencies; and sixth, given that NAD^+ concentrations in cells tend to be low, the effectiveness of any exogenous glycolytic substrate that requires NAD^+ for its oxidation, including glucose, FBP and G3P, may be limited by a limited NAD^+ supply unless exogenous NAD^+ is provided along with the added substrate.

The invention also includes providing compositions comprising NAD^+. Because NAD^+ is required for metabolism of either FBP or G3P to produce ATP, the combination of NAD^+ and FBP or the combination of NAD^+ and G3P delivered to tissue, for example in a metabolically deficient tissue, is more effective than adding FBP or G3P or NAD^+ alone.

3-PG and 2-PG

The invention further comprises augmenting generation of ATP with 3-phosphoglycerate (3-PG). 3-PG is a reaction product of G3P. 3-PG occurs downstream of G3P in the metabolic pathway after the consumption of NAD^+ and the production of 1 ATP. 3-PG is capable of producing 1

ATP, but requires no NAD^+ and therefore produces no NADH. Because no NADH is made, conversion of the pyruvate formed from added 3-PG to lactic acid will deplete NADH and thus limit further lactate production. Assuming glycolytic intermediates upstream of 3-PG are depleted under anoxic conditions, NADH will not be regenerated from G3P oxidation, and therefore pyruvate thereafter made from the addition of 3-PG will not be further metabolized into lactic acid. Instead, much 3-PG metabolized under anaerobic conditions must end up as pyruvate which is ready for oxidation by the mitochondria. Since no lactate is generated in this process, the inhibition due to lactate and the cycling of $NADH/NAD^+$ are avoided. Thus, embodiments of the invention comprise delivering a composition comprising 3-PG to tissue such as tissue in a metabolically deficient state.

The compound following 3-PG in the glycolytic pathway, 2-phosphoglycerate (2-PG), is less stable and thus less suitable for practical use. Because either 2-PG or 3-PG produce only 1 ATP molecule, as compared to 2 ATP molecules produced by each G3P, twice as much 2-PG or 3-PG as G3P should be used.

2,3-DPG

A conversion of 3-PG to 2-PG is necessary for the production of ATP from 3-PG. 2,3-diphosphoglycerate (2,3-DPG) catalyzes the conversion of 3-PG to 2-PG and is therefore useful, especially in combination with 3-PG. The conversion reaction of 3-PG to 2-PG consumes no ATP molecules. However, the formation of 2,3-DPG by the cell requires ATP and thus in ischemic conditions, where ATP is in short supply, providing 2,3-DPG will conserve ATP for other cellular purposes.

Since the metabolism of either FBP or G3P has as an intermediate 3-PG, providing 2,3-DPG in addition to either FBP or G3P will likewise help to conserve ATP molecules. The invention therefore embodies supplementing a metabolically deficient tissue with 2,3-DPG to help

glycolysis to proceed, for example when endogenous 2,3-DPG is depleted, perhaps due to lack of ATP.

Since either NAD^+ or 2,3-DPG could be rate limiting, bottlenecks of the glycolytic pathway may be avoided by providing both of these compounds concurrently. The invention provides compositions including these compounds and delivery of these compositions to tissue, for example to a metabolically deficient person or in vitro tissue.

The invention also encompasses compositions and administration of FBP or G3P with NAD^+ and 2,3-DPG. When FBP and G3P are also provided along with NAD^+ and 2,3-DPG, glycolysis will not be impeded by lack of substrate or by limitations of these cofactors.

Phosphoenol Pyruvate (PEP)

The compound that follows 2-PG in the glycolytic pathway is phosphoenol pyruvate (PEP). PEP gives rise to the second ATP produced in glycolysis and is sufficiently stable to be practical to use. The present invention embodies this use by administrating PEP to tissue. For example, 2,3-DPG is not required for the conversion of PEP to pyruvate. The invention thus comprises delivering PEP to tissue, especially wherever or whenever 2,3-DPG is limiting or the enzymes that convert 3-PG to PEP are inhibited. Similar to 3-PG, PEP will not consume NAD^+ and NADH will not be produced. PEP is therefore a particularly advantageous substrate. Metabolism of PEP to pyruvate also produces 1 ATP molecule, so 2 PEP molecules will replace 1 G3P molecule or 1/2 FBP or glucose molecule.

Pyruvate with FBP

FBP can also be used in conjunction with pyruvate, one of its metabolites. This combination as used in the present invention can be especially useful in an intra-ischemic treatment approach, for example, treatment of brain tissue after the onset of ischemic insult but before reoxygenation. When used intra-ischematically, the FBP component of the combination will allow ATP production to

begin immediately, before reoxygenation, while the added pyruvate will be immediately available to the citric acid cycle once oxygen is reintroduced. Practicing the invention by using this composition comprising FBP and pyruvate to treat brain ischemia should provide greater assurance of hippocampal protection than the use of FBP alone.

Significant quantities of FBP can be converted into lactate, but the invention's provision of an exogenous pyruvate pool will reduce conversion of this lactate to pyruvate upon reoxygenation, thus making more pyruvate available for immediate mitochondrial metabolism to produce ATP in the citric acid cycle while simultaneously conserving NAD^+ that would otherwise be consumed by lactate oxidation and thereby avoiding inhibition of FBP oxidation by NAD^+ depletion. Slowing the rate of lactate conversion to pyruvate will slow the rise in intracellular pH postanoxically, helping to avoid the pH paradox.

In treatment of a complex tissue, organ or organism according to the invention, the combination of FBP and pyruvate offers a further advantage in that cells relatively impermeable to either FBP or pyruvate will gain protection from the presence of the alternate fuel source in the compound.

Co-enzyme A

Before pyruvate can be utilized in the citric acid cycle it must be decarboxylated and the resulting acetate moiety combined with coenzyme A (CoA) to form acetyl Coenzyme A (acetyl CoA). This process is irreversible, effectively precluding conversion of pyruvate to lactate. Although CoA is nominally impermeable, the inventor has found that CoA improves ion pumping in kidney slices. This predicts that CoA can penetrate cells and mitochondria under conditions of deep hypothermia or thereafter. CoA penetration during periods of ischemia at temperatures closer to normal metabolic temperatures allows pyruvate to be irreversibly converted to acetyl CoA

in the mitochondrial matrix even in the absence of oxygen. Less lactate will therefore be produced, and NAD^+ otherwise used for conversion of lactate to pyruvate will be conserved for other purposes. At the same time, the mitochondria will be primed by the presence of acetyl CoA for rapid ATP production upon reoxygenation. This invention thus provides for delivering CoA to a tissue to protect tissues or cells thereof from death, by augmenting ATP production during and after ischemia, while also sparing NAD^+ for other metabolic purposes.

The inventor has found CoA to be particularly effective for restoring viability of kidney tissue formerly exposed to high concentrations of cryoprotective agents. As shown in Figure 1, exposure to high concentrations of cryoprotectants results in a reduction in the ability to synthesize ATP after removal of the cryoprotectant and restoration of normal conditions for metabolism. Also as shown in Figure 1, this reduction in ATP synthetic capacity is related to transport of potassium and sodium which in turn reflects tissue viability. The inventor has found that exposure to cryoprotective agents apparently results in a blockade of glycolysis that persists after cryoprotectant washout, possibly due to the induction of sulfhydryl oxidaton, but that, surprisingly, CoA administered after cryoprotectant washout can seemingly reverse this blockade, perhaps by facilitating pyruvate removal and oxidation while simultaneously acting as a reducing agent to stimulate pyruvate kinase and inhibit fructose 1,6-bisphosphatase. The ability of CoA to restore viability after exposure to cryoprotectants is not known in the prior art.

<u>NAD^+ Plus CoA and Other Combinations</u>

The oxidation of pyruvate involves the formation of an enzyme-hydroxyethyl complex upon decarboxylation of pyruvate followed by an oxidation step to produce an enzyme-acetyl complex. This oxidation step cannot proceed unless NAD^+ is available. Once the enzyme-acetyl complex is formed, it can react with reduced coenzyme A to form

acetyl CoA. Thus, NAD^+ is indirectly required for the CoA-dependent removal of pyruvate and its ultimate conversion to acetyl CoA.

The invention therefore provides a composition including CoA and NAD^+ for delivery to tissue, for example to an ischemic individual or organ. NAD^+ is necessary for ATP production, for example, from oxidation of G3P under anaerobic conditions. Delivering CoA plus NAD^+ will avoid depletion of NAD^+. As an extra advantage, since both cofactors, NAD^+ and CoA, each inhibit lactate accumulation, this combination of the cofactors is especially effective in preventing lactate accumulation.

CoA and NAD^+ are effective at producing ATP when substrate fuel is present. Thus a combination of CoA with NAD^+, and a fuel source, for example G3P, is especially preferred. Another composition and method embodied in the invention therefore comprises providing a composition including NAD^+, CoA and a fuel source such as G3P to prevent lack of cofactors or fuels from limiting ATP production.

The inventor has found that, though NAD^+ and CoA by themselves are effective, the combination of NAD^+ and CoA sometimes results in no effect. Because NAD^+ is an oxidizing agent and CoA is a reducing agent, CoA may reduce NAD^+ to NADH and NAD^+ may oxidize CoA (which has a free sulfhydryl group, often represented as CoASH) to oxidized CoA (CoASSCoA), with the result that neither of the products are effective for producing ATP. This antagonism can be avoided by mixing NAD^+ and CoA less than 30 minutes before use, by administering them from separate containers to avoid contact between them until they are in the biological system at hand, or by cycling between NAD^+ and CoA administration. For cycling, since anoxia produces reducing conditions, NAD^+ should normally be given first to maintain glycolysis and to provide the NAD^+ that is needed to allow CoA to react with the acetate moiety produced from pyruvate by the pyruvate dehydrogenase complex. Approximately 5-60 minutes after

NAD^+ administration, and more preferably 10-50 minutes after NAD^+ administration (or if hypoxia/anoxia lasts less than these times, then immediately upon reoxygenation), CoA can be given to convert the produced lactate and pyruvate into acetyl CoA, thereby facilitating postanoxic mitochondrial energy production and reducing lactate and pyruvate accumulation so that glycolysis can proceed. For cold storage of cells, tissues and organs, embodiments of the invention feature exposure to NAD^+ during storage and to CoA at the end of storage or after 12-24 hours of cold storage. For cryopreservation of cells, tissues and organs, other embodiments of the invention feature exposure to NAD^+ prior to cooling to cryogenic temperatures and to CoA after washout of the cryoprotectant.

CoA plus Carnitine

Ischemia leads to the release of free fatty acids from cell membranes into the cytoplasm or extracellular matrix. Fatty acids can participate in reperfusion injury through conversion to inflammatory mediators as well as through lipid peroxidation related damage to cells once oxygen supply is reestablished. By restoring fatty acids to membranes or by promoting the translocation into mitochondria of these fatty acids, this toxicity may be avoided.

CoA is used for intracellular transport of fatty acids for subsequent metabolism. Fatty acids are activated on the outer mitochondrial membrane by the formation of fatty acyl CoA. The fatty acyl group is then transferred to carnitine and the complex then crosses the inner mitochondrial membrane to the matrix area where lipid metabolism proceeds. CoA and carnitine thus mitigate ischemic damage by removing proinflammatory, peroxidizable lipids from the cytoplasm while also contributing to aerobic ATP production by facilitating delivery of fatty fuels to mitochondria.

To improve ATP metabolism following hypoxia or ischemia, embodiments of the invention therefore involve

delivering CoA plus carnitine to accomplish, among other functions, removal of damaging fatty acids from the cytoplasm. The invention, through delivery of CoA and carnitine for fatty acid removal, minimizes production of inflammatory mediators, such as prostaglandins, from fatty acids.

Acetyl-carnitine has been shown to be beneficial in reversing cerebral ischemic damage. However, acetyl-carnitine is converted to carnitine in the mitochondria. The invention thus includes delivering acetyl-carnitine and/or carnitine with CoA to facilitate transport of fatty acids to the mitochondria matrix for conversion to acetyl CoA. Providing carnitine to ischemic regions, for example 0.1-100 mM carnitine or acetyl carnitine, in combination with 0.1-100 mM CoA in a wash or intravenous solution, so as to provide a concentration of about 0.1-10 mM carnitine or acetyl carnitine and 0.1-5 mM CoA in fluid that is directly in contact with the distressed cells or tissue, can help mitigate ischemic damage by participating in removal of fatty acids during hypoxia and facilitating their oxidation after reoxygenation.

The value of CoA in facilitating this function of carnitine through provision of properly activated fatty acids has not been previously suggested. Providing carnitine or acetyl-carnitine alone may have limited effectiveness due to the slowness or absence of the required activation of fatty acids before their transport across the inner mitochondrial membrane. For example, reports of benefits from acetyl-L-carnitine have not been successfully reproduced. The invention thus provides for delivery of a composition containing carnitine and CoA to a metabolically deficient person or tissue. Providing a combination of CoA and carnitine or acetyl-carnitine therefore provides CoA for activation of the fatty acid and a carnitine compound for translocation to the mitochondrial matrix. The combination thus increases the effectiveness in reversing ischemic damage over provision of either compound alone. Once again, mitochondria will

be primed, this time by the presence of activated fatty acids, for production of ATP upon reoxygenation.

The combination of CoA and carnitine is not apparent from the prior art for at least three reasons. First, CoA has been traditionally considered impermeable to living cells. The inventor has, as documented herein, provided the first evidence that exogenously supplied CoA can enter cells and produce a therapeutic effect. Second, activation of fatty acids by CoA is an ATP-dependent process. To deplete precious ATP under anoxic conditions by activating fatty acids for transport to reduce inflammation after reoxygenation is in direct opposition to the provision of glycolytic intermediates to produce ATP anoxically to prevent cell death prior to reoxygenation. However, in the inventor's estimation, the amount of ATP so consumed is sufficiently limited to prevent this problem from being lethal if ATP-boosting agents are used simultaneously with CoA and carnitine during anoxia, or if CoA and carnitine are made available just prior to or shortly after reoxygenation. Third, the prior art does not indicate carnitine to be limiting for ATP production or cell\organ viability during or after ischemia or cryopreservation.

G3P plus NAD^+ plus Acetyl CoA

Since most fuel molecules enter the citric acid cycle as acetyl CoA, direct provision of acetyl CoA may also be advantageous as a fuel source. This substrate will then be available for utilization in the citric acid cycle immediately upon reoxygenation. Since the formation of acetyl CoA from either pyruvate or fatty acids requires NAD^+, numerous enzymes, coenzymes, and a redox cycle involving the formation and breakage of disulfide bonds at the active site of one of the enzymes, direct provision of acetyl CoA may avoid metabolic bottlenecks due to an insult to any one or more of these systems, for example, an insult resulting from a chemical or ischemic attack. Furthermore, acetyl CoA is probably the most rapidly

available and energy-efficient compound, gram for gram or mole for mole, that exists in the body.

Although acetyl CoA will be highly efficient at restoring ATP immediately upon reoxygenation, it will not produce any ATP under anoxic conditions. In fact, acetyl CoA is a negative regulator of pyruvate kinase, which converts PEP into pyruvate. Although inhibition of this step will preclude lactate production, it will also halve the efficiency of glycolytic energy production during anoxia. For this reason, a preferred approach would be to use G3P + NAD^+ to drive ATP production during hypoxia and to introduce acetyl CoA only when sufficient oxygen is or becomes available to allow conversion of most added acetyl CoA to CoA + CO_2 so that pyruvate kinase is not inhibited or so that inhibition of pyruvate kinase is more than offset by ATP production from acetyl CoA.

Therefore, delivery of exogenous acetyl CoA during or shortly before reoxygenation, or of G3P + NAD^+ during anoxia followed by acetyl CoA during or just before reoxygenation according to the invention provides immediate access to acetyl CoA for production of ATP upon reoxygenation and, if needed, continuous energy production during hypoxia as well. Delivery of acetyl CoA according to the invention may be carried out as an alternative to delivery of pyruvate for any application in which pyruvate may be useful, for example, salvage of hippocampal neurons or other tissues after an ischemic insult.

Situational Examples

Compounds and compositions described herein for maintaining and augmenting ATP production and preventing accumulation of harmful substance will be most advantageous when given during continuing conditions of limited oxygen supply or effectiveness, e.g., under conditions such as drowning, hemorrhagic shock, cardiac arrest or mitochondrial poisoning with agents such as cyanide.

In most such situations, immediate restoration of full tissue oxygenation cannot be accomplished quickly,

and cyanide poisoning cannot be reversed immediately. Therefore a therapeutic window for improving a patient's energy metabolism generally appears between the time a patient can be treated and the time oxidative metabolism can be restored by improving tissue oxygenation and/or removing poisons that interfere with energy metabolism.

Although ischemic attacks often occur without warning, there are many instances of planned ischemia, for example, ischemia necessary for organ transplantation. The invention provides for greater transplantation success, either by increased survival of an organ or improved function of an organ after transplantation by modulating ATP production in the donor organ during its ischemic period. The donor organ can be treated by perfusion, washing and/or bathing with ATP augmentation compounds or compositions of the invention, before removal of an organ from the donor and/or before transplantation into the host. The invention embodies uses of compositions and compounds of the invention before and/or during cryopreservation or cold storage, during transport, and/or during and/or after surgery. The viability of tissue during and following storage will be improved. Return of function after reperfusion will also be accelerated.

Cryopreservation

Cryopreservation often causes generalized cellular damage that must be repaired by energy dependent mechanisms. Methods of the invention provide cofactors and substrates for maintenance of ATP levels critical to repairing cryopreservation induced damage. Cryopreservation in the absence of dimethyl sulfoxide leads to damage that may be addressed by pre-freeze and/or by post-thaw administration of ATP-producing agents. For example, in an especially preferred embodiment, G3P + NAD^+ are administered prior to resumption of aerobic metabolism (i.e., before warming to temperatures that permit full mitochondrial function), preferably where G3P and NAD^+ are administered in preferred concentrations of about 0.1 to

20 mM. Cryopreservation in the presence of dimethyl sulfoxide may be successfully addressed in another preferred embodiment of the invention by administration of CoA after removal of the dimethyl sulfoxide and prior to and/or following mitochondrial activation by warming. In both embodiments, further benefits may be obtained by introducing acetyl CoA near the time of warming to temperatures that permit mitochondrial function.

Reversing Anesthesia

The invention is also useful for reversal of effects of anesthesia. Restoration of normal metabolism is essential for reversing anesthetic effects. The compounds, compositions and methods described herein are useful for reversing anesthesia, for example, barbiturate induced anesthesia or Hypnorm induced anesthesia, by increasing the metabolic supply of ATP.

Prolonged Storage of Blood

Presently, the most commonly transplanted tissue is blood. Several modalities have been developed for preserving blood up to several weeks or months in an unfrozen condition. In these modalities, conditions that stimulate cellular synthesis of ATP and 2,3-DPG have been found to extend the useful transplantable shelf life of blood. The invention embodies methods that improve storage of blood and blood products by providing solutions and combinations of ATP augmenting agents described herein to provide superior ATP maintenance results over those obtained using existing solutions.

Since red blood cells (RBCs) have no mitochondria, the compounds and compositions described herein which involve only mitochondrially mediated benefits will only be useful for non-RBC cellular blood products. The non-mitochondrial effects, however, will also serve to maintain ATP levels in stored blood which includes RBCs. Thus admixing of compounds and compositions of the invention with blood allows further extension of the shelf life of blood and blood products.

Alternatives to Insulin and Glucose for Diabetics

Another metabolic disorder requiring maintenance of ATP levels is unavailability of glucose resulting from diabetes. In "insulin-independent" (type II) diabetes, glucose is not transported into cells due to insulin resistance, and therefore is not utilized effectively for ATP production by the cells. Since glucose is a preferred fuel source for producing ATP, especially in neuronal tissue, ATP levels fall. This dearth of ATP can result in coma or death. The invention provides treatment for an individual in such a situation by delivering to the individual compounds and compositions of the invention as alternatives to glucose for producing ATP. In insulin-dependent (type I) diabetes, exogenous insulin is used to compensate for a deficient endogenous insulin production, but an excessive dose of exogenous insulin may cause blood glucose levels to decrease excessively as glucose is transported from the blood into the cells. In this case, blood glucose levels may fall so much that patients may die from lapsing into an irreversible coma due to lack of circulating glucose available to brain cells. Practice of the invention permits hypoglycemic diabetic coma to be reversed by infusion of the compounds and compositions of the invention to achieve rapid restoration of cerebral ATP production in the face of severe hypoglycemia. This practice will often be more effective than glucose infusion alone due to the inefficiency of ATP production from glucose and the actual drop in cellular ATP that may occur when glucose is first administered.

As an alternative or supplement to insulin treatment for providing fuel by stimulating glucose intake by the cells, the invention provides an alternative substrate fuel, for example, FBP, to partially replace glucose as the energy source. The patient can then be restored to a state of relative normalcy of energy metabolism despite the failure of the tissues to take up a normal amount of glucose. This alternative treatment will be preferable to insulin treatment alone when insulin is

so ineffective, for example in insulin resistance, that adequate glucose uptake is simply impossible in response to exogenous insulin or when the required insulin dose is unacceptably high, for example, when the required insulin does would be more damaging than elevated glucose levels.

When an excess of insulin has been given, use of the alternative fuel sources and other ATP augmentation and maintenance compounds and compositions of the invention may be useful, optionally in combination with glucose, to prevent or reverse diabetic coma or to prevent or reverse clinical death associated with hypoglycemia due to the inadequacy of glucose by itself to generate energy with sufficient speed.

The amount of fuel source needed by an individual may be calculated by multiplying either the normal molar quantity of glucose needed for healthy life per unit time or the effective glucose deficiency expressed in the same units by the stoichiometry described herein, (e.g., about 0.5 to 1 for FBP, 1 to 2 for G3P, and 2 to 4 for PEP) and delivering the compounds or compositions of the invention at the resulting calculated rate until the diabetic coma or other indication resolves. In a preferred embodiment, this delivery should permit approximately 0.1 to 10 mM levels of the ATP-promoting substance(s) in the plasma to be achieved.

Both insulin dependent and insulin resistant diabetics will be helped by the provision of the alternate fuels according to the invention.

Other Uses

Methods of the invention also provide for use of ATP augmentation and maintenance compounds and compositions of the invention compounds as dietary supplements for individuals who have chronic ischemic states, for example, pulmonary insufficiency (resulting from, for example, lung cancer, pulmonary edema, occupational exposure or aging), poor peripheral or cardiac circulation, or phlebitis. These methods can also be of value to other individuals who may simply feel

fatigued. Compounds and compositions of the invention can be prepared as tablets, capsules, powders, liquids or in other dosage forms as dietary supplements and when desired may be enterically coated. Compounds and compositions of the invention may be provided in any suitable form for administration, as can be selected by one of ordinary skill in the art without undue experimentation. For example, they may be delivered orally, intravenously, intraperitoneally, intramuscularly or as suppositories in appropriate pharmaceutically acceptable carriers and forms.

During aging, cellular housekeeping does not keep up with the accumulation of damage to organelles and cells. Although age related defects in mitochondrial ATP production are not well defined, aging persons experience a sense of fatigue and lack of energy as their years progress. The invention alleviates aging effects in part by modulating ATP production to normal or supernormal levels. This stimulating effect should provide a generalized improvement in function which may combat a part of the generalized decline in functions characteristic in aging.

Another method of embodiments of the invention involves use of compounds and compositions of the invention for sports competitions. Sporting competitions may require maximal physical performance. A maximally exercising athlete will typically produce considerable lactic acid in his or her muscles, with a consequent fall in intracellular pH. This has an immediate effect of reduced performance and may also produce muscle soreness during days subsequent to exercising which may impact future training and/or performance. By ingesting or otherwise receiving compounds or compositions of the invention, for example, NAD+, FBP, G3P, 3-PG and/or PEP, prior to exercise, the athlete will be able to produce more ATP in relatively hypoxic and acidotic muscles than he or she could otherwise produce given the inhibition of glycolysis from decreased pH and subnormal oxygen

tensions. The use of 3-PG and/or PEP by athletes may be particularly advantageous for preventing the accumulation of lactic acid in muscles when these agents are administered in sufficient dosage to compete with glucose as an energy source. The resultant decrease in lactate production will reduce lactate mediated muscle soreness and acid mediated performance limitations. CoA, acetyl CoA, and these agents in combination with about 0.1 to 2 mM dichloroacetate (which activates pyruvate dehydrogenase) will also tend to reduce lactate accumulation.

The invention also includes timed release dosage forms of ATP augmenting compounds, for example, FBP, G3P, 3-PG or PEP, especially for use in endurance sports, but also for other uses requiring continual metabolic support (including recovery from trauma, surgery, etc.). There are no known endogenous mechanisms for controlling blood levels of these compounds. Timed release dosage forms of the invention will provide steady levels of these compounds for a long duration of effectiveness. Timed release formulations may also be especially advantageous for treating diabetes or for treating effects of aging. Timed release formulations of pharmaceutical compounds and compositions are well known to those skilled in the art, and appropriate formulations can be selected and prepared without undue experimentation.

Dosages of the various cofactors used in the invention will vary depending on the form of delivery, for example, bathing, injecting or oral ingestion and permeabilities of the tissues to be treated. Effective amounts of ATP substrates and co-factors can be determined by routine experimentation similar to experiments in the examples in the instant application. Generally, intracellular concentrations of these factors of 50 μM or less will be effective and will be produced by extracellular concentrations ranging from about 0.1 mM to 10 mM. Depending on temperature and permeabilities of the compounds relative to the specific cell membrane,

concentrations of each ATP substrate or co-factor may be adjusted for specific applications.

When a compound or composition of the invention is delivered in an oral or circulating form, blood or perfusate levels of the ATP substrates and co-factors will be adjusted to provide intracellular concentrations determined to be effective.

EXAMPLES

Example 1

Rabbit kidney cortical slices (0.5 mm thick) were kept anoxic (bubbled with 100% N_2 at pH 7.4) for 45 min, then were reoxygenated (100% O_2, pH 7.4) for 45 min and ATP content was determined. The substrates listed for each condition were the only ones available during both anoxia and hyperoxia. Table 1 shows that fructose 1,6-bisphosphate (FBP) is no more effective than glucose at enabling ATP synthetic capacity, perhaps because of the high pH of the anoxic medium, which may allow glycolysis to proceed without acid blockade. More importantly, G3P was as effective as either glucose or FBP despite being present at only a 2 mM concentration, which is only 1/10th of its stoichiometrically appropriate concentration of 20 mM (two times the FBP concentration). G3P was thus 5 times more effective than either glucose or FBP and 20 times as effective as pyruvate on a molar basis.

TABLE 1

Substrate Available	Concentration	ATP Content (micromoles/mg wet weight)
Glucose	10 mM	0.443±.019
Fructose 1,6-bisphosphate	10 mM	0.431±.097
Glyceraldehyde-3-phosphate	2 mM	0.437±.054
Pyruvate	40 mM	0.420±.050

Example 2

In Example 2, pH was held at 7.0 and oxygen was not resupplied. The anoxic period was 60 min. This protocol allows ATP maintenance in the anoxic state to be

examined directly at a pH closer to the low pH prevailing during complete anoxia in vivo; in vivo, access to exogenous substrate implies some circulation which implies a pH slightly higher than the 6.5-6.9 often seen with complete blockage of flow. In Table 2 results are expressed in micromoles ATP per mg of dry weight of the solid pellet spun down after homogenization of the tissue slices (rabbit kidney).

The results again show that FBP is no better than glucose, that 2 mM G3P is approximately as effective as 10 mM glucose or 10 mM FBP, and that phosphoenolpyruvate (PEP) is by far more effective than either glucose or FBP when PEP is used at its proper stoichiometric concentration. The results from the combination of NAD^+ with G3P suggesting that NAD^+ may be inhibitory appear to be an artifact due to interference of NAD^+ with the enzymatic assay used to measure ATP concentration (Figure 2). NAD^+ is a product of the reaction by which ATP is measured, and sufficient NAD^+ to inhibit the ATP detection reaction by endproduct inhibition was probably carried into the assay medium from the unwashed slices.

Separate experiments in which PEP was added to ATP in the ATP assay indicated no false detection of PEP as ATP, i.e., PEP does not produce a false positive assay for ATP. ATP production from pyruvate was surprisingly high, given that pyruvate is not a glycolytic fuel, but pyruvate, as expected, gave the lowest ATP yield of all substrates other than the artifactual result from G3P + NAD^+.

Selected results of this experiment are summarized also as "Exp. 1" of Figure 3.

TABLE 2

Substrate Available	Concentration	ATP Content (micromoles/mg dry pellet weight)
Glucose	10 mM	0.465±.015
Fructose 1,6-bisphosphate	10 mM	0.475±.005
Glyceraldehyde-3-phosphate	2 mM	0.415±.0015
G3P plus 20 mM NAD^+	2 mM	0.300±.050
Phosphoenolpyruvate	40 mM	0.720±.06
Pyruvate	40 mM	0.345±.005

Example 3

The conditions of Example 3 were similar to those of Example 2, but 3-phosphoglycerate (3-PG) was used in place of pyruvate, G3P concentration was raised to a stoichiometrically equivalent value relative to FBP of 10 mM, and the anoxic period was 60 min. rather than 45 min. Table 3 shows the results of Example 3, wherein for unknown reasons, the assay results are all low in comparison to the other examples. As expected from the first two experiments, G3P exceeds glucose in its ability to enable ATP production, the ATP content being about 70% higher than with glucose. Consistent with Example 2, NAD^+ gave the lowest apparent ATP content, undoubtedly again for artifactual reasons (interference with the assay). Also consistent with Example 2, PEP yields a higher ATP content than glucose, the PEP result being 52% higher than the glucose result. Also, 3-phosphoglycerate, which was not tested in the other examples, exceeds glucose-stimulated ATP by 22%. Inconsistent with Examples 1 and 2 is a finding that FBP now yields more ATP than glucose and, in fact, yields more ATP than PEP. Nevertheless, FBP did not consistently outperform glucose whereas PEP did; and G3P results showing an improvement over glucose when used at the proper stoichiometry are consistent with what would be expected based on its parity with glucose when used at drastically sub-stoichiometric concentrations. In summary, all substrates tested in Example 3 were more effective than glucose, provided NAD^+ did not disrupt the assay.

Selected results of this experiment are also summarized pictorially as "Exp. 2" of Figure 3.

TABLE 3

Substrate Available	Concentration	ATP Content (micromoles/mg dry pellet weight)
Glucose	10 mM	0.115±.005
Fructose 1,6-bisphosphate	10 mM	0.300±.02
Glyceraldehyde-3-phosphate	20 mM	0.195±.005
G3P plus 20 mM NAD$^+$	20 mM	0.055±.009
Phosphoenolpyruvate	40 mM	0.175±.015
3-Phosphoglycerate	40 mM	0.140±.020

Example 4

Example 4 shows that exposure to the test substrates improves ATP synthetic ability after reoxygenation. The conditions of Example 4 were similar to those of Example 3, except liver slices were used and the slices were transferred after a 60 min period of anoxia to the fresh oxygenated media that contained only glucose as the energy source. These conditions better simulate the in vivo state, in which the primary substrate available upon reoxygenation will be glucose. These conditions also allow NAD$^+$ to be tested under anaerobic conditions and then removed afterwards so as to avoid interference with the ATP assay. ATP was measured after 150 min of incubation in the presence of oxygen. The results are striking. Although the variability in the control group (glucose) is high, the mean ATP content in the glucose control group is higher than the mean for FBP, indicating that FBP provided no protection during 60 min of anoxia in comparison to glucose. In fact, the result for FBP is indistinguishable from the result for pyruvate, one of FBP's glycolytic metabolites, again indicating no protection by FBP. In contrast to these two conventional groups, all of the substrates of the present invention tested herein give mean ATP yields in excess of that obtained for glucose. As in Example 2, PEP outperforms G3P, although marginally. Most strikingly, the G3P + NAD$^+$

group gave ATP contents averaging 83% higher than the glucose group, and 49% higher than the G3P group without NAD^+, thus indicating that NAD^+ is indeed a highly effective additive for driving anaerobic ATP production. These NAD^+ results confirm the inventor's interpretation concerning the earlier technical difficulties of measuring the effects of NAD^+ on ATP content.

TABLE 4

Substrate Available	Concentration	ATP Content (micromoles/mg wet pellet weight)
Glucose	10 mM	0.333±.163
Fructose 1,6-bisphosphate	10 mM	0.303±.099
Glyceraldehyde-3-phosphate	20 mM	0.409±.054
G3P plus 20 mM NAD^+	20 mM	0.608±.002
Phosphoenolpyruvate	40 mM	0.428 (n=1)
Pyruvate	10 mM	0.292±.046

Example 5

This experiment was similar to that described in Example 4, but was carried out on kidney slices rather than on liver slices. The data are summarized in Figure 4. 10 mM G3P and 10 mM G3P plus 20 mM NAD^+ were far superior to glucose in sustaining, during 60 min of anoxia, the ability to recover ATP synthetic capacity postanoxically. Pyruvate in addition to G3P did not help beyond the effect of G3P alone. Another positive treatment group was 20 mM PEP plus 20 mM pyruvate, which was equivalent to 10 mM G3P, although scatter in this group requires more samples to achieve statistical significance. The combination of PEP and pyruvate is attractive due to the relatively low cost of PEP and pyruvate in comparison to G3P. Surprisingly, the protective effect of PEP alone was, at best, minimal. However, the small number of samples in this group (2) may have prevented an effect from being seen.

Example 6

Figure 5 summarizes the effects of several experiments involving the storage of kidney slices (rabbit

renal cortex) at about 0°C in an effective storage solution. Addition of 2 mM CoA to the solution raised viability as indicated by an achieved K/Na about 30% greater in the CoA group after 4 days of storage, converting the K/Na ratio to a value approximating what is normally seen in control (unstored) slices. Either 2 or 20 mM NAD^+ had a similar protective effect on cellular viability during simple cold storage of kidney slices. Different symbol shapes refer to separate experiments.

Example 7

Figure 6 shows that, surprisingly, when either G3P or PEP were used in an attempt to reverse the ATP deficit, and hence the K/Na deficit produced by VS41A, as described in Figure 1, the results were negative. 10 mM G3P was not effective and 20 mM PEP was actually detrimental. The notations indicate the these agents were present in the assay medium used to determine K/Na (Cross solution, or "CS" (100.1 mM NaCl, 1.5 mM NaH_2PO_4, 40.2 mMKCl, 6.1 mM sodium acetate, 5.0 mM dextrose, 0.8mM $CaCl_2$; π=290; pH=7.4), described in Fahy, Cryo-Letters, 1984) as well as in the cold storage solution used after cryoprotectant washout and before exposure to CS ("0%"). The failure of these agents to improve K/Na is surprising and suggests that pyruvate kinase, which is required for producing ATP from PEP, is blocked, preventing any benefit of PEP and reducing any benefit of G3P. Blockage of pyruvate kinase would convert G3P into PEP energetically speaking (1 ATP per mole vs. 2 ATPs per mole) and would convert PEP into an inhibitor of G3P breakdown, explaining why PEP is counterproductive in this instance. These effects are believed to be due to the dimethyl sulfoxide (DMSO) present in VS41A (3.1 M DMSO, 3.1 M formamide, 2.21 M 1,2-propanediol; pH=7.0 at R.T.) (for discussion of the cryoprotectant formula, see Fahy, da Mouta et al., 1995, in The Cell Biology of Trauma, Lemasters and Oliver, eds., CRC press, Boca Raton, FL) and hence are not expected to be present in the case of cryoprotectant formulae devoid of DMSO.

To determine whether this blockade could be overcome by facilitating pyruvate removal, effectively encouraging pyruvate kinase activity by removing the end product of the reaction, the experiment was repeated using 1 mM dichloroacetate (DCA) in the CS after washout (WO) of VS41A or in both the CS and the VS41A washout solutions. In neither case was any statistically significant effect seen (Figure 7). DCA activates pyruvate dehydrogenase phosphatase, which dis-inhibits pyruvate dehydrogenase by dephosphorylating it.

Because the combination of G3P and NAD^+ was frequently superior to G3P alone in anoxia or in anoxia/reoxygenation experiments, and because of the possibility that 1 mM DCA was insufficient after VS41A exposure, the experiment shown in Figure 8 was carried out, in which G3P was combined with NAD^+ prior to incubation at elevated temperatures in CS (20 mM G3P was present after VS41A washout only, whereas 2 mM NAD^+ was present in the VS41A washout solutions and after completion of WO) and with the presence of 4 mM DCA in the CS. The results show a statistically significant but moderate improvement.

Figure 9 shows a) that the improvement seen in Figure 8 can be obtained using 2 mM NAD^+ alone, but b) that exposure to 2 mM CoA after washout of the cryoprotectant (VS4, a dilution of VS41A to 89% of full strength) eliminated fully half of the injury associated with cryoprotectant exposure, a striking and highly novel result.

Figure 10 shows that when CoA is administered after slices have been exposed to cryoprotectant, cooled to -30°C, and then returned to normal conditions, the normal detrimental effect of cooling shown in the figure can be effectively reversed. This may be a reversal of cooling injury, but more likely it is a reversal of the toxicity of the cryoprotectant, confirming the results shown in Figure 9, that has the net effect of negating the additional injury associated with cooling.

Figure 11 shows that when CoA is present simultaneously with the cryoprotectant, 2 mM CoAdoes not reverse cryoprotectant toxicity.

While not wishing to be bound by any theory, the inventor speculates that the latter result is because the cryoprotectant may be able to oxidize CoASH to CoASSR and because the effect of CoA depends on being in the reduced (CoASH) form. The inventor further speculates that reduced CoA may act by reversing DMSO-induced SH oxidation in the pyruvate dehydrogenase complex, in pyruvate kinase, and in fructose 1,6-bisphosphatase as well as by providing CoASH to accept the acetyl moiety formed from pyruvate by the pyruvate dehydrogenase complex and thereby permit acetyl-CoA to be synthesized and pyruvate dehydrogenase to become able to form additional acetyl moiety from pyruvate.

In summary, Examples 1 through 7 amply demonstrate that anaerobic substrates that have previously been used as glucose substitutes are not the most effective anaerobic substrates that can be used. Contrary to the known art, substrates other than FBP and pyruvate can yield higher ATP production during anaerobic and subsequent aerobic exposures than can FBP or pyruvate, and that the cofactors NAD^+ and Coenzyme A can play critical roles in facilitating ATP synthesis and protection of cell viability after a variety of ATP-depleting insults, either when applied alone or in combination with novel acting ATP-promoting substrates and compositions including novel acting ATP-promoting substrates disclosed herein.

While the invention has been described with reference to particular preferred embodiments, the invention is not limited to the specific examples given, and other embodiments and modifications can be made by those skilled in the art without departing from the spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. A process for augmenting ATP production in an oxygen metabolism impaired tissue, comprising:

delivering to the tissue at least one exogenous compound selected from the group consisting of 3-PG, G3P, PEP, NAD^+, coenzyme A and acetyl coenzyme A.

2. A process according to claim 1, wherein the tissue is in a hypothermic state.

3. A process according to claim 2, further comprising delivering exogenous pyruvate to the tissue.

4. A process according to claim 1, wherein said delivering of said at least one compound follows administration of an anesthetic to said tissue, said at least one compound being delivered in an amount of effective to reverse effects of said anesthetic.

5. A process according to claim 1, wherein the tissue is a blood product.

6. A process according to claim 1, wherein the tissue is in an individual suffering from a chronic ischemic state.

7. A process according to claim 1, wherein said tissue is part of a living mammal, comprising delivering the at least one compound to said mammal prior to exercise.

8. A process according to claim 1, wherein said delivering is effected during a period when the tissue experiences ischemia or anoxia.

9. A process according to claim 8, wherein the tissue is part of a cryopreserved tissue and said delivering is effective to produce ATP augmentation that assists in repairing cellular damage in said tissue.

10. A process according to claim 1, wherein said delivering is effected a predetermined time before the tissue is subjected to an anticipated period of ischemia or anoxia.

11. A process according to claim 1, further comprising delivering exogenous FBP to said tissue.

12. A process according to claim 1, wherein the at least one compound comprises NAD^+.

13. A process according to claim 1, wherein said at least one compound comprises NAD^+ and G3P.

14. A process according to claim 1, further comprising delivering exogenous 2,3-DPG to said tissue.

15. A process according to claim 1, wherein said at least one compound comprises NAD^+, said process further comprising delivering to said tissue at least one other exogenous compound selected from the group consisting of FBP, G3P, 3-PG, PEP, dichloroacetate and pyruvate.

16. A process according to claim 1, wherein said at least one compound comprises NAD^+, said process further comprising delivering exogenous 2,3-DPG to said tissue.

17. A process according to claim 1, wherein said at least one compound comprises NAD^+, and said process further comprises delivering to said tissue at least one of said coenzyme A and acetyl coenzyme A.

18. A process according to claim 17, further comprising delivering to said tissue exogenous carnitine or exogenous acetyl carnitine.

19. A process according to claim 1, wherein said at least one compound comprises NAD^+, said process further comprising delivering to said tissue exogenous 2-3-DPG and at least one exogenous compound selected from the group consisting of 3-PG, G3P and FBP.

20. A process for mitigating damage due to an oxygen and/or fuel deficit in a cryopreserved organ, comprising:

delivering to the organ at least one compound selected from the group consisting of FBP, 3-PG, G3P, PEP, NAD^+, coenzyme A, acetyl coenzyme A, carnitine and acetyl-carnitine.

21. A process for treating a patient afflicted with a glucose metabolism defect, comprising:

administering to the patient at least one compound selected from the group consisting of FBP, 3-PG, PEP, NAD^+, coenzyme A and acetyl coenzyme A in an amount

effective to treat symptoms of said glucose metabolism defect.

22. A process according to claim 21, wherein said amount is effective to prevent or reverse diabetic coma or hypoglycemic associated clinical death.

23. A method for improving athletic performance, comprising administering to an individual before an athletic competition at least one compound selected from the group consisting of FBP, 3-PG, G3P, PEP, NAD^+, coenzyme A in an amount effective to augment ATP levels in said individual during said competition.

24. A method according to claim 23, wherein said at least one compound is administered in a timed release form.

25. An ATP augmentation composition, comprising at least one compound selected from the group consisting of FBP, G3P, 3-PG, PEP, NAD^+, pyruvate, coenzyme A and acetyl coenzyme A in a timed release form.

26. A composition according to claim 25, wherein said composition is in a form suitable for oral administration.

27. A process for augmenting ATP production in an organ, comprising delivering to said organ exogenous pyruvate and FBP.

28. An ATP augmentation composition comprising:

a pharmaceutically acceptable carrier; and

at least one compound selected from the group consisting of G3P, 3-PG, PEP, NAD^+, coenzyme A and acetyl coenzyme A.

29. A composition according to claim 28, further comprising at least one compound selected from the group consisting of pyruvate and FBP.

30. A composition according to claim 28, further comprising at least one member selected from the group consisting of carnitine and acetyl carnitine.

31. A composition according to claim 28, wherein said at least one compound comprises NAD^+ and a second compound selected from said group.

32. A composition according to claim 28, wherein said at least one compound comprises (i) NAD^+ and (ii) coenzyme A or acetyl coenzyme A, said composition further comprising carnitine or acetyl carnitine.

33. A composition according to claim 28, wherein said composition comprises (i) NAD^+, (ii) at least one compound selected from the group consisting of coenzyme A and acetyl coenzyme A, and (iii) at least compound selected from the group consisting of G3P, 3-PG, PEP, pyruvate and FBP.

34. A composition according to claim 28, wherein said composition comprises (i) NAD^+, (ii) at least one compound selected from the group consisting of coenzyme A and acetyl coenzyme A, (iii) at least one compound selected from the group consisting of G3P, 3-PG, PEP, pyruvate and FBP, and (iv) at least one compound selected from the group consisting of carnitine and acetyl carnitine.

35. A method for maintenance of ATP production in an individual comprising:

delivering to said individual a composition according to claim 23, said individual internalizing said composition and distributing said composition to at least one tissue of said individual by at least one route selected from the group consisting of circulation, digestion and absorption through skin or membranes.

36. A process according to claim 1, wherein said process comprises at least one member selected from the group consisting of: reducing or preventing tissue damage in acute or chronic ischemia, reversing anesthesia, treating diabetes, reversing or preventing coma, reversing processes of ageing, supplementing a diet, enhancing athletic performance, improving outcome of tissue transplantation or other surgery, mitigating ischemic and/or hypoxic or anoxic conditions, and preserving cold stored or cryopreserved transplantable tissues.

37. A process according claim 1, wherein said tissue is or is about to be impaired in its oxygen metabolism.

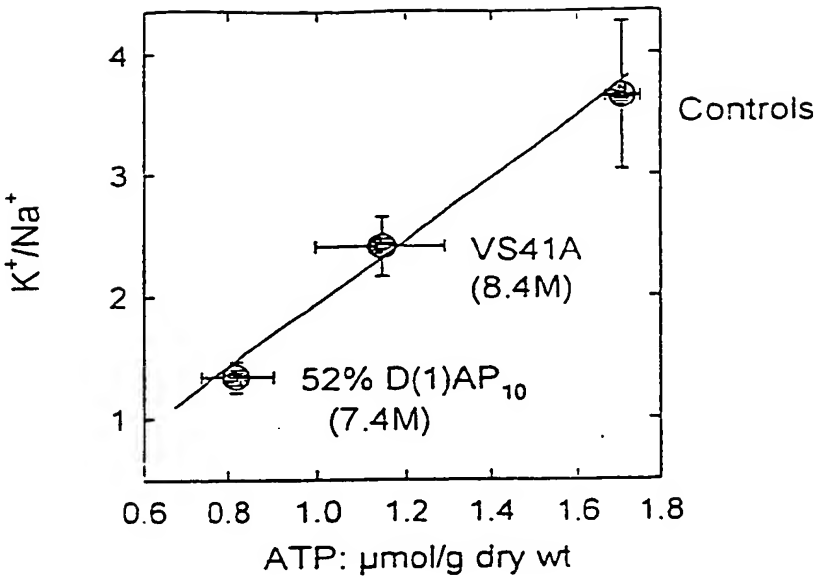


FIGURE 1

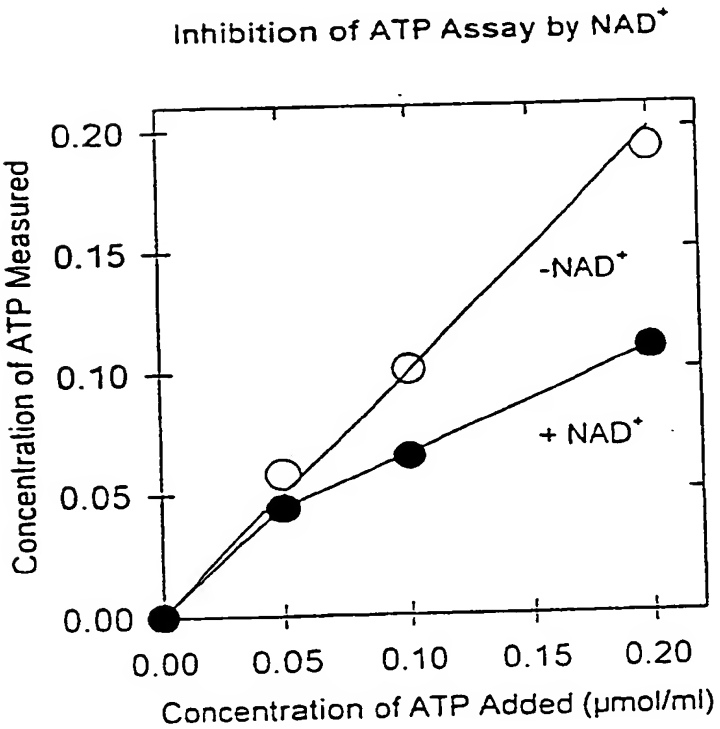


FIGURE 2

Synthesis of ATP Under Anoxic Conditions
(100% N_2 for 60 min, pH 7.0; rabbit renal cortex)

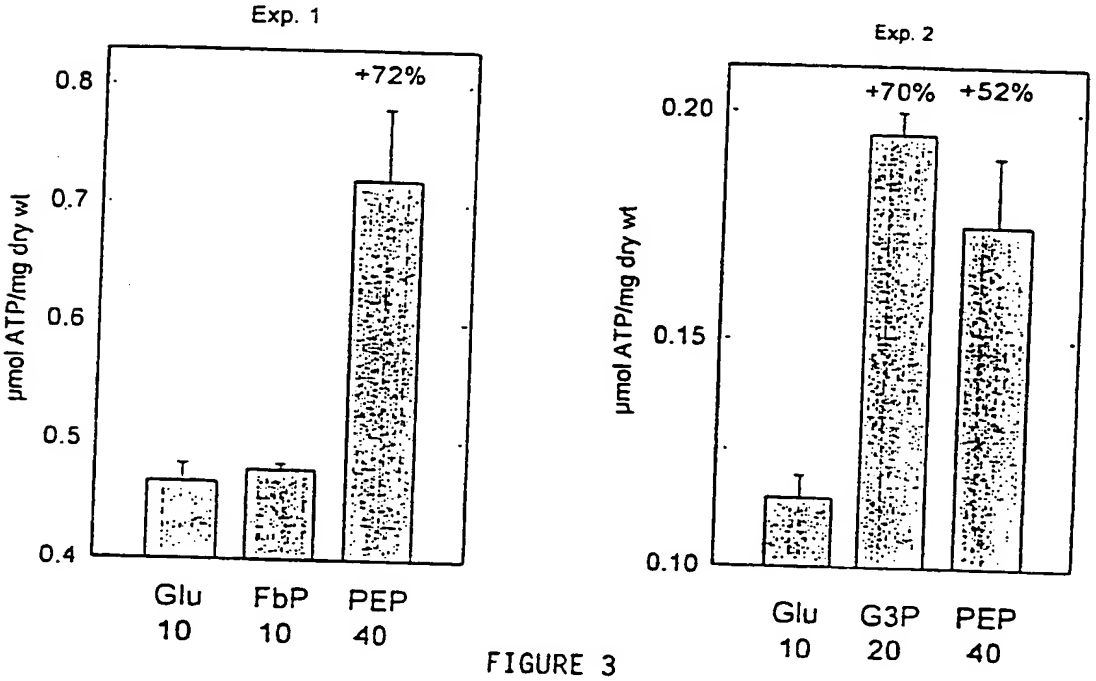


FIGURE 3

Postanoxic Synthesis of ATP: Rabbit Renal Cortex

(100% N_2, 60 min, pH 6.9, then
100% O_2, 90 min, pH 7.4)

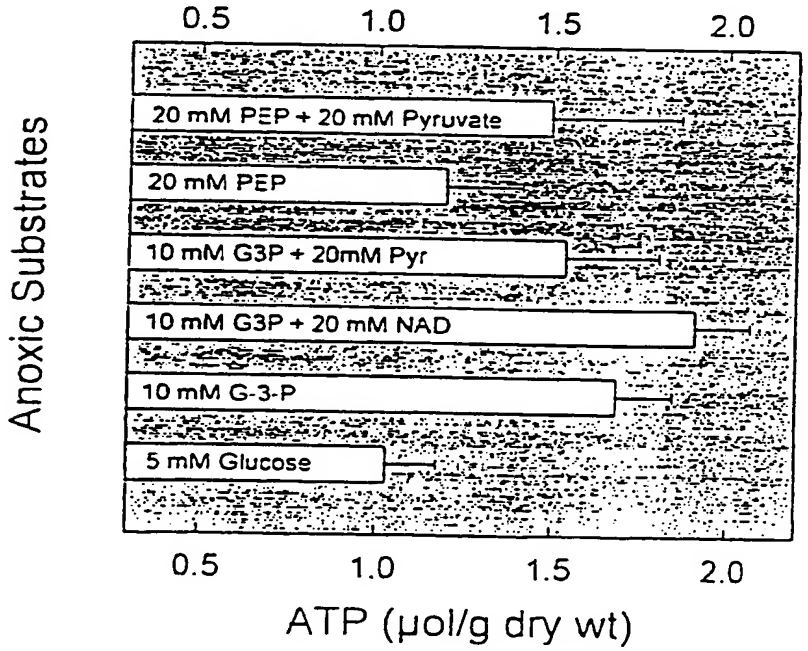


FIGURE 4

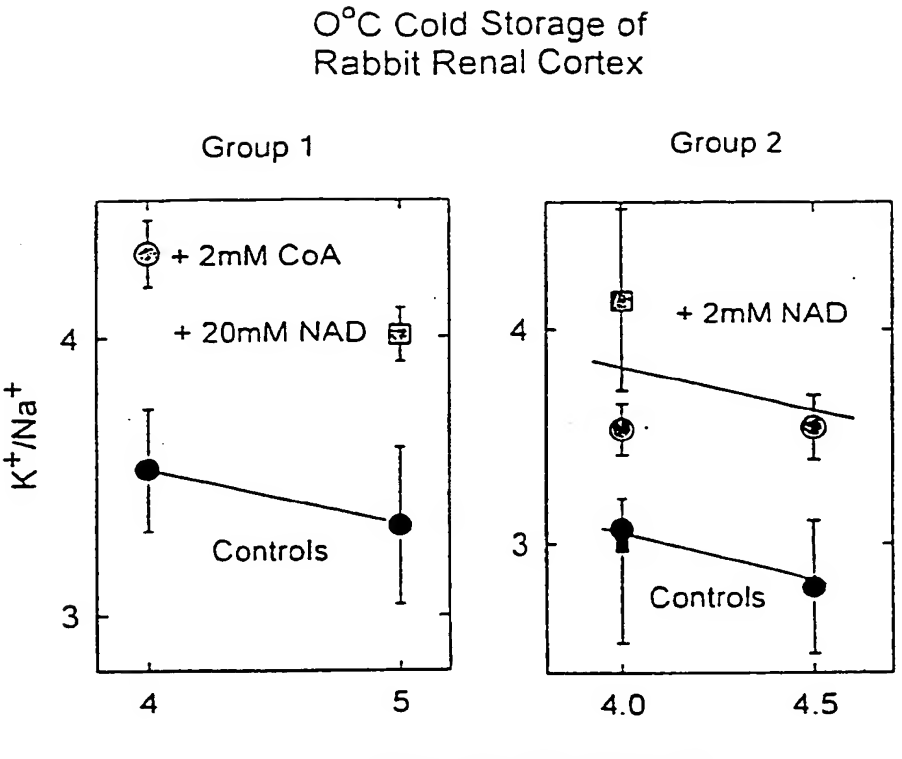


FIGURE 5

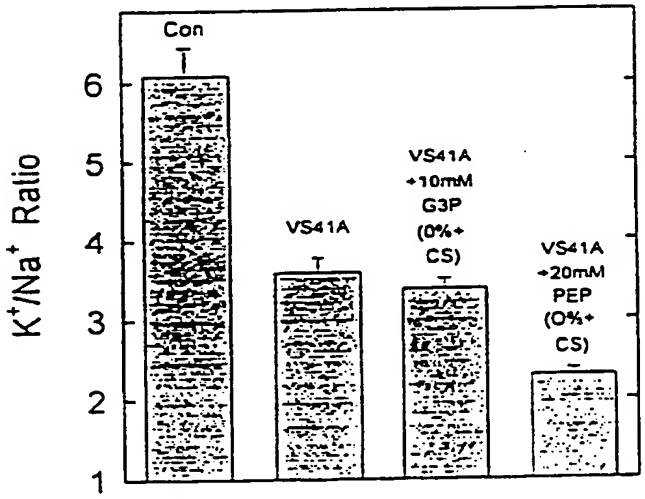


FIGURE 6

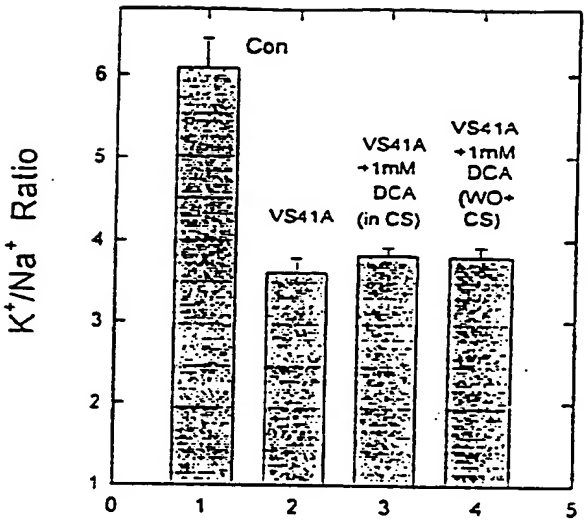
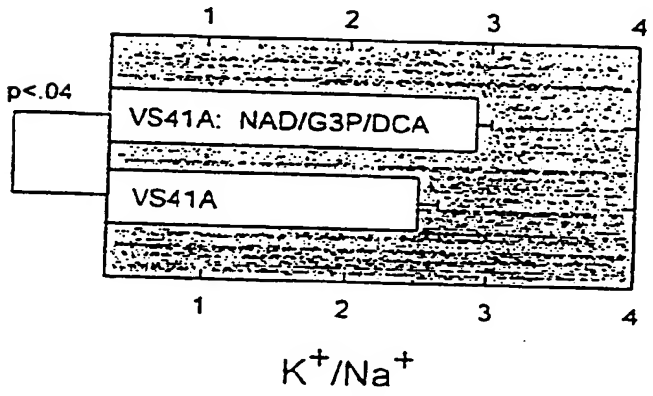


FIGURE 7

Resistance of VS41A Toxicity to Direct ATP Augmentation



20 mM G3P (0,0+); 2 mM NAD (±); & 4 mM DCA (CS)

FIGURE 8

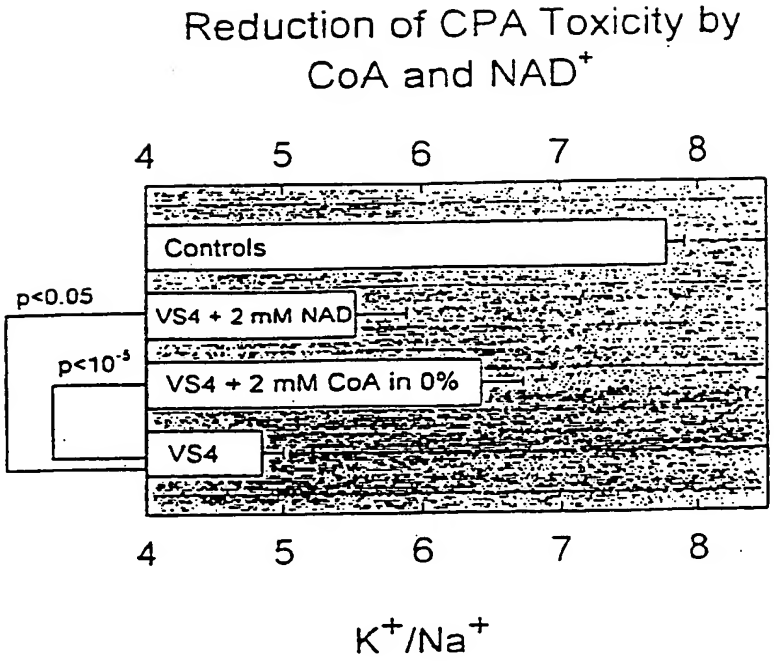


FIGURE 9

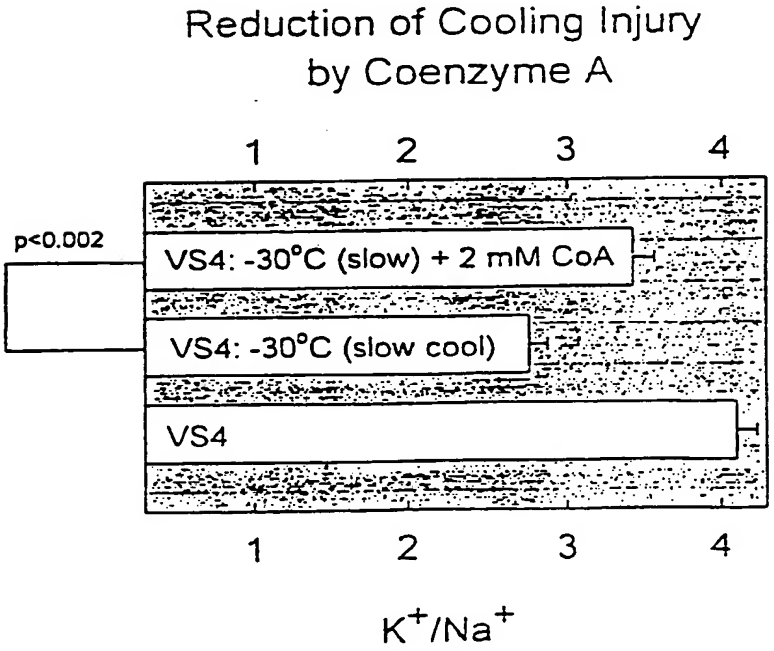


FIGURE 10

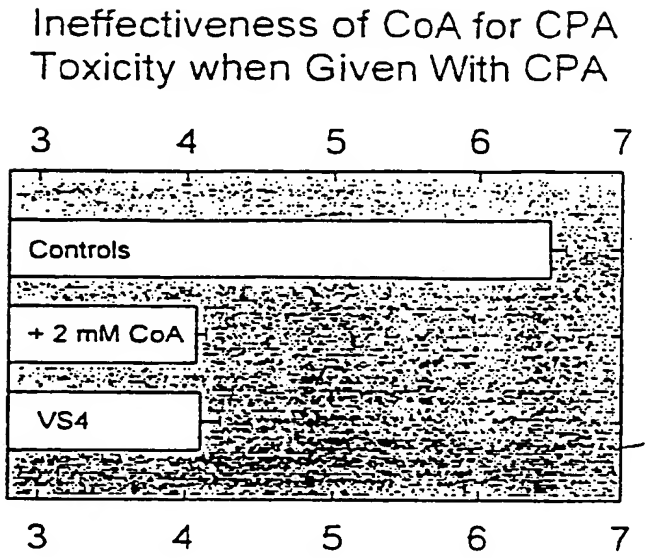


FIGURE 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10255

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/70, 31/115
US CL :514/23, 43, 45, 694
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/23, 43, 45, 694

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS,
search terms: ATP(production, augmentation, increasing)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,546,095 A (A. K. MARKOV) 08 October 1985, entire document.	1-37
Y	US 4,703,040 A (A. K. MARKOV) 27 October 1987, entire document.	1-37
Y	US 4,757,052 A (A. K. MARKOV) 12 July 1988, entire document.	1-37
Y	US 5,039,665 A (A. K. MARKOV) 13 August 1991, entire document.	1-37
X	US 5,395,822 A (Y. IZUMI ET AL) 07 May 1995, column 4, lines 43-52 and column 10, lines 62-67 - column 11, lines 1-14.	25 and 26

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 30 AUGUST 1996	Date of mailing of the international search report 26 SEP 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JOHN W. ROLLINS Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10255

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Merck Index, 12th Ed. 1966, (Whitehouse Station, NJ.), page 417, column 2, No. 2531, Coenzyme A.	28
X	Merck Index, 12th Ed. 1966, (Whitehouse Station, NJ.), page 763, column 2, No. 4491, Glyceraldehyde 3-Phosphate.	28
X	Merck Index, 12th Ed. (Whitehouse Station, NJ.) page 1088, column 2, No. 6429, NAD.	28